Evolutionary Systematics. 9 2025, 33–38 | DOI 10.3897/evolsyst.9.138575

PENSOFT

EVOLUTIONARY SYSTEMATICS

On a new haematophagous species of the genus *Leptoconops* (*Holoconops*) Kieffer (Diptera, Ceratopogonidae) from the coastal region of West Bengal, India

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Academic editor: Karina L. Silva-Brandão ♦ **Received** 4 October 2024 ♦ **Accepted** 22 January 2025 ♦ **Published** 25 February 2025

Abstract

Leptoconops (*Holoconops*) *beccus* **sp. nov.** is described from India after molecular and morphological data. Both sexes of the new species were fetched from Digha, the coastal region of West Bengal. A revised key to the Oriental species of the subgenus *Holoconops* Kieffer is also designed.

Key Words

Biting midges, *Holoconops*, Key, New species, Oriental region

Introduction

Ceratopogonidae is one of the largest and most diversified families in the superfamily Chironomoidea of the infraorder Culicomorpha. This family is further divided into three extant subfamilies: Ceratopogoninae Newman, Forcipomyiinae Lenz, and Leptoconopinae Noè (Borkent et al. 2022). Around 6300 living species belonging to 109 different genera are distributed around the world (Borkent 2024). All the haematophagous species in the Ceratopogonidae are found in three genera *Leptoconops* Skuse, 1889, *Culicoides* Latreille, 1809, *Austroconops* Wirth & Lee, 1958, and the subgenus *Lasiohelea* Kieffer, 1921 of the genus *Forcipomyia* Meigen, 1818 (Borkent and Spinelli 2007). Members of this family are well-known for their medico-veterinary importance (Lee et al. 1963; Yeh and Chuang 1996; Chen et al. 2005). The genus *Leptoconops*, an extremely nasty biter, is usually found in locations near the coast (Clastrier and Wirth 1978). *Leptoconops* are widely distributed biting midges in the world with a total of 174 species belonging to seven different subgenera (Borkent and Dominiak 2020). Among them, the Oriental realm registered 17 species from four different subgenera, *Leptoconops* s. str., *Holoconops* Kieffer, 1918, *Proleptoconops* Clastrier, 1974, and *Styloconops* Kieffer, 1921 (Borkent and Dominiak 2020; Mazumder et al. 2010). Immature stages are generally found in sandy banks, saline marshlands, protected sandy beaches, and depressions adjacent to the coast (Clastrier and Wirth 1978). The larvae consume the organic material of sand, which includes a diverse range of microorganisms (Wirth and Atchley 1973).

The presence of *Leptoconops* midges often becomes a major nuisance due to their aggressive diurnal biting activity and their effect on the tourism industry in the coastal area. According to Kettle (1962), only females have the blood-sucking tendency, and their bites may transmit infections to humans and animals. Although bites of *Leptoconops* spp. can cause intense itching for months and also lead to infection and serious outcomes, the bites' immediate effects are generally thought to be less severe than the lesions that may develop later (Whitsel and Schoeppner 1966; Aussel 1993). The *Mansonella* Faust, 1929 is a human filarial nematode transmitted by tiny blood-sucking midges like *Culicoides* spp. and *Leptoconops* spp. (Ta-Tang et al. 2018) and the filarial disease Mansonellosis was reported from Bihar, India

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(Kumari et al. 2017). The mansonellosis primarily affects underprivileged communities residing in tropical and subtropical regions (Lima 2016). Although mansonellosis is not now the focus of the National Filaria Control Programme from India, it would be a concern with the expansion of the disease in endemic areas in the near future.

The main objective of this article is to describe a new species of the subgenus *Holoconops*. We provided DNA barcoding of the mitochondrial COXI gene of the proposed new species. A revised Oriental key to the adult males and females of the subgenus *Holoconops* Kieffer is also provided.

Material and methods

All the specimens were collected from coastal area of West Bengal, India by using various types of light traps like UV light trap (27W), CFL bulb (40 W) and Filament bulb (60W) in the month of October, 2023. Collected specimens were preserved in 70% ethanol. A small fragment of tissue from the ventral part of the thorax and one set of legs from one of the male specimens was taken for DNA extraction, using Qiagen DNeasy Blood and tissue kit. Universal primers LCO1490 (5′–GGTCAACAAATCATAAAGATATTGG–3′) forward primer and HCO2198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) reverse primer (Folmer et al. 1994) were employed for amplification of the COI region, following mostly the procedure of Harrup (2018). The amplified products were observed in a 1% agarose gel using electrophoresis and sent out for bidirectional Sanger's sequencing (3730 xl DNA Analyzer, Hitachi). The obtained sequences were uploaded to the NCBI GenBank. The specimens were mounted in glass slides following Wirth and Marston (1968). For morphology and terminology of adults, we mostly followed Borkent et al. (2009) and Santarm et al. (2023) whereas terminology of antennal sensilla followed after Wirth and Navai (1978). All the measurements were taken in micrometres (μm), except for the width and length of the wing, which were measured in millimetres (mm). The ranges are followed by the mean value in parentheses. The number of specimens encountered in the study is indicated by 'n'. The proposed new species is compared with allied or closely related species following relevant literature. However, we have not examined any type specimens for comparison. The images were captured using a fluorescent microscope with a camera attached, under bright field conditions (Leica DM 1000, Germany). Illustrations were produced via a compound microscope (Wild Leitz GMBH, Portugal) attached to Camera Lucida. The illustrations were inked with Rotring Isograph (Germany) 0.1 and 0.2 pens. The pen drawings were scanned at a resolution of 1200 dpi using an HP Scanjet 200 to create digital copies of the drawings in either TIFF or JPG format. These copies were then edited in Adobe Photoshop CS3 to make the figures for the plates.

Abbreviations with their full forms. **AR**—Antennal ratio, **PR**—Palpal ratio, **P/H**–Proboscis to head ratio, **WL**—Wing length, **WW**—Wing width, **CR**—Costal ratio; **TR**—Tarsal ratio, **HTC**—Hind tibial comb.

All the types are presently kept at the Biosystematics and Entomology Division, Department of Zoology, The University of Burdwan, Burdwan, West Bengal, India, and will be subsequently deposited in the National Zoological Collections (NZCI), Kolkata, in due course.

Key to the species of the subgenus *Holoconops* Kieffer from the Oriental realm

(Modified after Mazumdar et al. 2010)

Male

1 Parameres jointed 2
– Parameres separate 4
2(1) Distal parts of parameres with spine-like projection ..... *L.* (*H.*) *xuthosceles* Chanthawanich & Delfinado, 1967
– Distal parts of parameres not spine like, variously shaped 3
3(2) Distal parts of parameres beak-like ..... *L.* (*H.*) *beccus* sp. nov.
– Distal parts of parameres triangular in shape ..... *L.* (*H.*) *ascius* Yu & Hui, in Yu 1988
4(1) Aedeagus broadly U-shaped, constricted basally ..... *L.* (*H.*) *brevistylus* Mazumdar, Saha & Chaudhuri, 2010
– Aedeagus rhomboid or club-shaped 5
5(4) Eyes hairy ..... *L.* (*H.*) *yunhsienensis* Yu, 1963
– Eyes bare 6
6(5) Aedeagus rhomboid in shape ..... *L.* (*H.*) *amplifemoralis* Chanthawanich & Delfinado, 1967
– Aedeagus club-shaped ..... *L.* (*H.*) *menglaensis* Liu & Yu, 1998

Female

1 Spermathecae without any hyaline punctation 2
– Spermathecae with hyaline punctation 7

2(1) Eyes hairy ..... *L.* (*H.*) *ascius* Yu & Hui, in Yu 1988
– Eyes bare 3
3(2) Spermathecae three ..... *L.* (*H.*) *yunnanensis* Lee, 1978
– Spermathecae two 4
4(3) Mandibular teeth more than 14 ..... *L.* (*H.*) *menglaensis* Liu & Yu, 1998
– Mandibular teeth 13 or less 5
5(4) Eyes separated by 11 facets ..... *L.* (*H.*) *beccus* sp.nov.
– Eyes separated by 10 facets 6
6(5) Mandible with 13 teeth; spermathecae oval shaped ..... *L.* (*H.*) *xuthosceles* Chanthawanich & Delfinado, 1967
– Mandible with 12 teeth; spermathecae roughly rounded ..... *L.* (*H.*) *amplifemoralis* Chanthawanich & Delfinado, 1967
7(1) Eyes hairy 8
– Eyes bare 9
8(7) TRIII less than two ..... *L.* (*H.*) *yunhsienensis* Yu, 1963
– TRIII greater than two ..... *L.* (*H.*) *laosensis* Clastrier, 1974
9(7) Eyes separated as wide as 13 facets; HTC with three spines ..... *L.* (*H.*) *brevistylus* Mazumdar, Saha & Chaudhuri, 2010
– Eyes separated by less than 13 facets; HTC with four spines 10
10(9) Eyes separated as wide as 10 facets; PR < 2.00 ..... *L.* (*H.*) *fortipalpus* Mazumdar, Saha & Chaudhuri, 2010
– Eyes separated as wide as six facets; PR > 2.00 ..... *L.* (*H.*) *tenebrostigmatus* Mazumdar, Saha & Chaudhuri, 2010

Taxonomy

Leptoconops (*Holoconops*) *beccus* sp. nov.

https://zoobank.org/092CC468-34F0-4B06-9E1E-0C93D8121C41

NCBI GenBank Accession no. PP358259.

Type material. ***Holotype*** ♂, India • West Bengal, Purba Medinipur, Digha (21.621513°N, 89.874962°E), 30.X.2023, Light trap, Col. L. Ghosh. ***Paratypes*** • 5♂♂ and 3♀♀, same data as holotype.

Diagnosis. The only species of the subgenus *Holoconops* from the Oriental realm that is diagnosed by the following combination of characters: **Adult male** (Fig. 1A–I). Gonocoxite wider at base to sub-basal part and narrow at anterior part; gonostylus short and stout, curved at middle and apex with two spines; parameres jointed at middle; posterior portion roughly rectangular, apex of paramere bearing an outwardly directed small projection; aedeagus elongate, rod-shaped, little swollen at apex. **Adult female** (Fig. 1J–R). Eyes bare; mandible with 12–13 teeth, palpomere III with 4–5 well developed protruded capitate sensilla; two unequal spermathecae without any hyaline punctation.

Description. Male (n = 6). ***Head.*** Head dark brown, eyes bare and separated about as wide as 10 facets. All flagellomeres (I–XII) small and globular except terminal flagellomere (XIII); proportion of length of flagellomeres (I–XIII): 18–20 (19.2): 09–10 (9.66): 08–11 (9.6): 08–11 (9.83): 08–11 (9.66): 08–10 (9.83): 07–10 (9.5): 08–11 (9.83): 08–12 (10): 10–15 (11.83): 10–15 (11.83): 11–20 (14.66): 36–45 (39.5); AR: 0.93–1.05 (0.98) (Fig. 1A). Maxillary palp with four palpomeres (terminal two fused); palpomere III (Fig. 1B) longest with a round, shallow sensory pit at apical end, with 4–5 protruding capitate sensilla; proportion of length of palpomeres (I–IV): 10–12 (11.3): 07–10 (8.6): 17–22 (20.5): 16–20 (18); PR 3.00–3.33 (3.1); P/H 0.62–0.64 (0.63).

Thorax. Dark brown. Scutellum with two bristles.

Wing (Fig. 1C). Covered with numerous microtrichia. WL 0.75–0.90 (0.82), WW 0.30–0.35 (0.32), CR 0.40–0.47 (0.45); stigma pale brown.

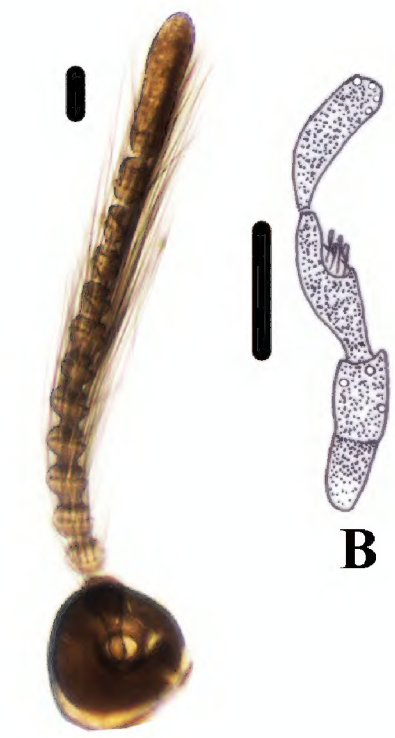
Legs. Uniformly brown to dark brown, without any banding pattern (Fig. 1D). HTC (Fig. 1E) with four spines and a spur; first one smallest, near spur and second one longest; first to fourth ones starting from spur measuring 20–25 (22.5), 42.5–45 (44.2), 30–37 (34), 25–32.5 (29.2) respectively. TRI 1.50–1.62 (1.56), TRII 1.38–1.51 (1.47), TRIII 1.25–1.40 (1.34).

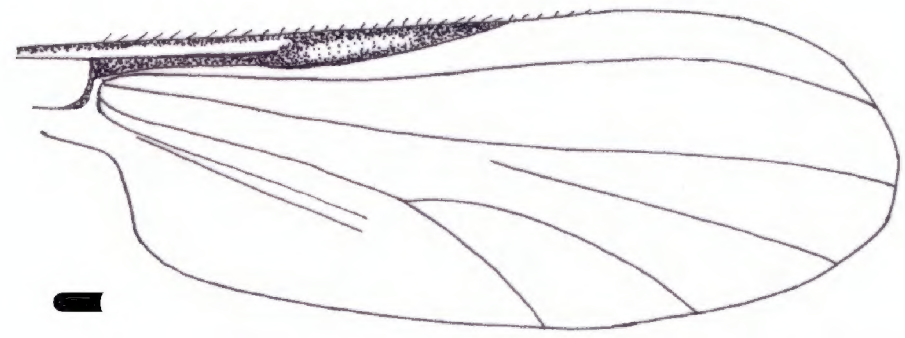
Abdomen. Dark Brown without any scale. Sternite IX 70–82 (75) long and 107–112 (109.6) wide, 1.4 times wider than length. Tergite IX 130–150 (140.5) long, 110–122 (117) wide, almost 1.2 times longer than wide and posterior margin triangular extending up to apex of Gonocoxite; basal margin of tergite IX bearing a outwardly directed, crescent-shaped greatly sclerotised structure with a sharply pointed inwardly directed spine at middle.

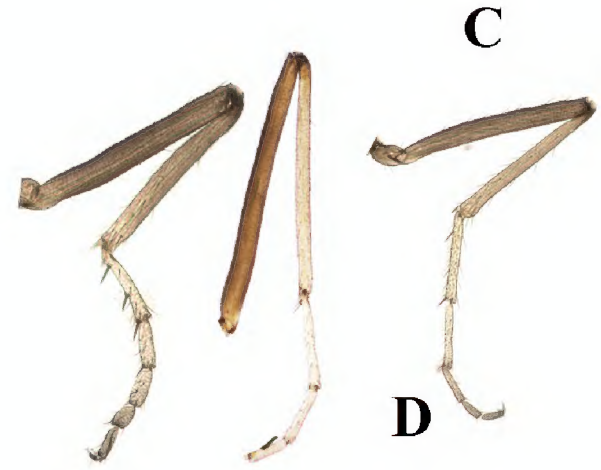
Genitalia (Figs. 1F–I). Apicolateral process small with 8–10 setae. Gonocoxite dark brown (Fig. 1F); wider at base and narrower in apex, 112–127 (119) long, almost twice its greatest breadth, 62–67 (63) with stout setae on inner part of gonocoxite. Gonostylus brown (Fig. 1G), 40–52 (46.5) long and 15–20 (18.33) wide, usually 0.4 times gonocoxal length, apex of gonostylus with two well-sclerotised equal-sized spines. Parameres (Fig. 1I) jointed at middle, basal part of each paramere roughly trapezoid in shape, apex of paramere with outwardly directed small projection, distal part each paramere boot-shaped. Aedeagus elongated, rod-shaped, little blunt at apex (Fig. 1H).

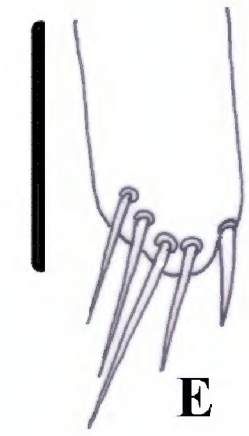
Female (n = 3). Overall, they have similar body colouration, and distribution of setae on body (Fig. 1J); similar shape of maxillary palp with males, hind tibial comb but some usual sexual and other differences are stated below.

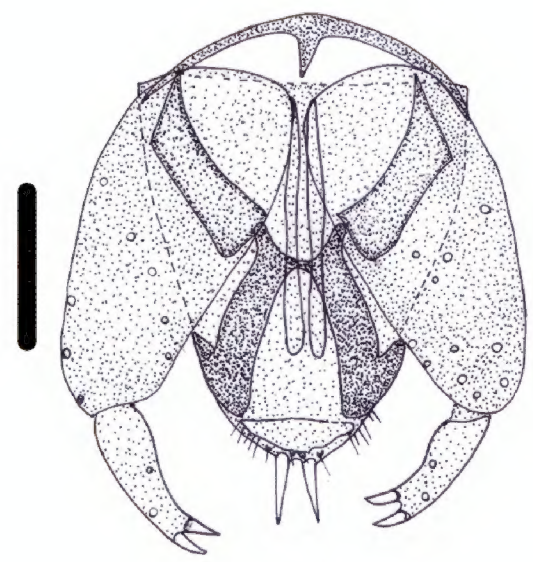
Head. All antennal flagellomeres rounded except terminal one (elongated) (Fig. 1K), pale brown to brown, bearing sensilla chaetica (I–X); terminal flagellomere (XI) with sensilla trichodea; proportion of length of flagellomeres

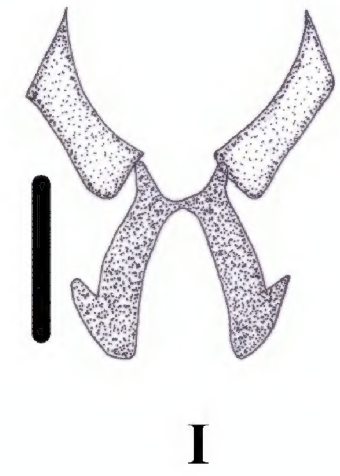


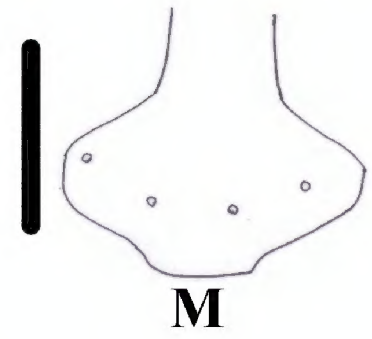


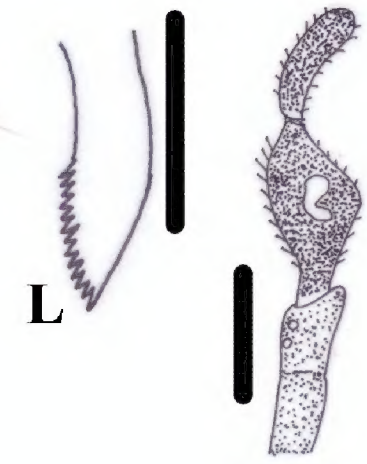


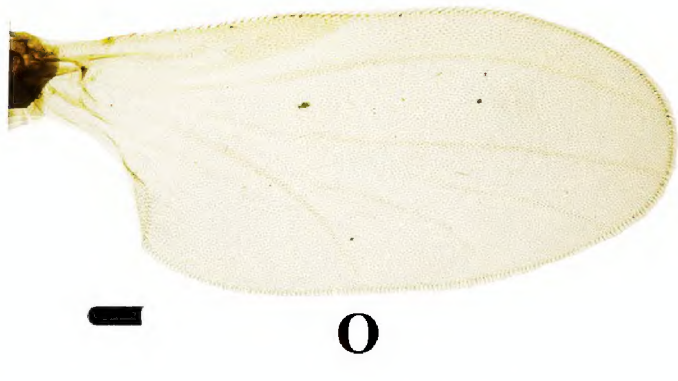


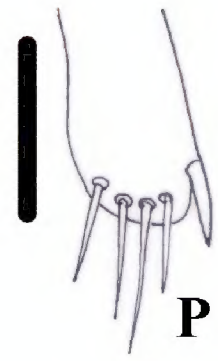


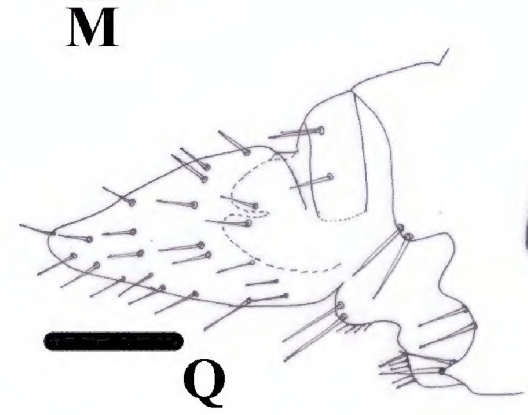












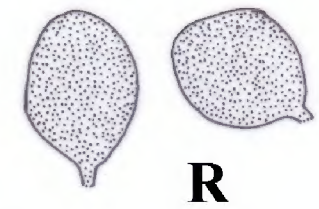


Figure 1. *Leptoconops* (*Holoconops*) *beccus* sp. nov. **A–I.** Male (♂): **A.** Antenna; **B.** Maxillary palpus; **C.** Wing; **D.** Hind, mid, fore legs (left to right); **E.** Hind tibial comb; **F.** Photograph of male genitalia (dorsal view); **G.** Illustration of male genitalia; **H.** Aedeagus; **I.** Parameres; **J–R.** Female (♀): **J.** Habitus (Blood engorged); **K.** Antenna; **L.** Mandibular teeth; **M.** Clypeus; **N.** Maxillary palpus; **O.** Wing; **P.** Hind tibial comb; **Q.** Genital lamella; **R.** Spermathecae. Scale bars: 0.05 mm.

(I–XI): 7–10 (8.33): 7–8 (7.33): 5–8 (6.33): 7–8 (7.33): 7–8 (7.33): 8–10 (8.66): 7–8 (7.33): 6–7 (6.66): 7–10 (8): 7–10 (8): 19–25 (21.33); AR 0.62–0.67 (0.64). Mandible armed, with 12–13 mandibular teeth (Fig. 1L). Labrum with five serrations. Clypeus with four setae arranged in a row (Fig. 1M). Proportion of length of palpomeres (Fig. 1N) (I–III, IV+V): 8–10 (9): 8–10 (9): 16–25 (21): 10–19 (14.66); PR 1.7–2.1 (1.9) and P/H 0.80–0.88 (0.83).

Thorax. Scutum and scutellum dark brown; scutellum with four bristles.

Wings (Fig. 1O). WL 0.77–0.90 (0.84), WW 0.36–0.42 (0.38) and CR 0.44–0.49 (0.46).

Legs. TRI 1.71–1.82 (1.77), TRII 1.33–1.51 (1.41), TRIII 1.40–1.48 (1.44); HTC with four spines and a spur (Fig. 1P); first to fourth spines from spur measuring 20–22.5 (21), 37.5–42.5 (39), 35–38 (36.5), 24–26 (25), respectively.

Abdomen (Figs. 1Q–R). Dark brown without any scales, with setae sparsely distributed. Genital lamellae pale brown (Fig. 1Q); little shorter, flat at base, and gradually narrow in apical part, 1.5 times longer than wide and roughly 2.5 times longer than sternite IX; sternite IX 55–63 (60) long and 162–175 (168) wide, well excavated. Spermathecae (Fig. 1R) two, oval, almost equal, well sclerotised, without any short neck and hyaline punctation; measuring about 40–47 (44.3) × 25–30 (27.3) and 36–44 (40.3) × 25–32 (28.3).

Remarks. The Indian species *Leptoconops brevistylus* Mazumdar, Saha & Chaudhuri, 2010 differs from *L. beccus* sp. nov. in separate parameres, nearly U-shaped aedeagus with basal ends tapered in male, and double rows of clypeal setae, HTC with three spines, and two unequal, oval to sub-oval spermathecae in female. Another Indian species *L. fortipalpus* Mazumdar, Saha & Chaudhuri, 2010 can be distinguished from the new species by double rows of clypeal setae, palpomeres I and II somewhat fused, lower PR (1.62) and unequal, oval spermathecae in female. Female members of *L. tenebrostigmatus* Mazumdar, Saha & Chaudhuri, 2010 can be separated from the new species in higher antennal ratio (>1), eyes separated by six facets, double rows of clypeal setae, palpomeres I and II not well separated, and higher PR (2.4).

The males of Nepalese species *L. amplifemoralis* Chanthawanich & Delfinado, 1967 differ in having the roughly rhomboid aedeagus, apex of parameres with a recurved spine and the absence of spines on the tergite band. The Chinese species *L. menglaensis* Liu & Yu, 1998 can be segregated from the new species in separate parameres, elongated gonocoxite and club-shaped aedeagus. The Thai species *L. xuthosceles* Chanthawanich & Delfinado, 1967 differs in swollen parameres in the middle and pointed recurved spine-like structure. The Chinese species *L. yunhsienensis* Yu, 1963 can be differentiated by separate parameres and hairy eyes. The females of *L. amplifemoralis* differ in round, equal spermathecae, and much longer and wider wing. Females *L. yunhsienensis* can be separated by hairy eyes and hyaline punctation in spermathecae. Females of *L. menglaensis* can be segregated from the new species by peach-shaped spermathecae.

At present, only three species of the genus *Leptoconops* have publicly available COI sequences. Two of these species are categorized in the subgenus *Leptoconops*, while the other falls under the subgenus *Styloconops*. Our proposed new species falls in the subgenus *Holoconops*. So at present, a molecular phylogenetic analysis may not yield fruitful results in the current scenario.

Etymology. The specific name of the new species "*beccus*" derives from the beak-like projection of the apex of parameres.

Distribution. India (West Bengal).

Bionomics. *Leptoconops* (*Holoconops*) *beccus* sp. nov. was procured from the Digha (6 m MSL), coastal plain of Purba Medinipur district, near the Bay of Bengal while the other three species [*L.* (*H.*) *brevistylus*; *L.* (*H.*) *fortipalpus* and *L.* (*H.*) *tenebrostigmatus*] were recorded mainly from the hilly area of the Chota Nagpur Plateau near the Mithon Water Reservoir (50 m MSL), Jharkhand, and Burdwan Town (30 m MSL), a mature delta of the Deltaic Proper of Gangetic West Bengal. The Nepalese species *L.* (*H.*) *amplifemoralis* Chanthawanich & Delfinado, 1967 was reported from Kathmandu (1300 m MSL), a part of Himalaya Biodiversity Hotspot. The Thai species *L.* (*H.*) *xuthosceles* was described from Chiang Mai (310 m MSL) near the Mae Ping River by Chanthawanich and Delfinado 1967. The biting midge *L.* (*H.*) *ascius* Yu and Hui, in Yu 1988 was described near the Lancang River (580 m MSL), Jinghong, Yunnan Province of the Oriental China. Both Oriental Chinese species, *L.* (*H.*) *menglaensis* Lee, 1978 and *L.* (*H.*) *yunnanensis* Liu & Yu, 1998 were reported from Mengla (600 m asl) of Yunnan Province. Another Chinese species *L.* (*H.*) *yunhsienensis* was described from Yunxian County (2000 m MSL) of Yunnan Province by Yu (1963). It was Clastier (1974) who described *L.* (*H.*) *laosensis* from the Nam Khan located on the left bank of the River Mekong (1800 m MSL), Laos. The new species in the subgenus *Holoconops* is only reported from the coastal area of the Oriental region.

Conflict of interest

On behalf of the co-authors, the corresponding author states that there is no conflict of interest.

Acknowledgements

We thank our Head, Department of Zoology, DST-FIST sponsored, The University of Burdwan for the laboratory facilities to conduct our research. We are also grateful to former Senior Technical Assistant Mr. Basudev Das, University Science Instrumentation Centre (USIC), University of Burdwan, for fabricating the Ultraviolet light traps and Junior Technical Assistant Mr Abhijit Roy of the same centre for assisting in taking some photographs from DM Leica 1000 (Bright field).

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